AD_____

Award Number: W81XWH-06-1-0365

TITLE: p63 in Development and Maintenance of the Prostate Epithelium

PRINCIPAL INVESTIGATOR: Sabina Signoretti, M.D.

CONTRACTING ORGANIZATION: Brigham and Women's Hospital
                                                    Boston, MA 02115

REPORT DATE: March 2007

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
                        Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01-03-2007	Annual	8 Feb 2006 – 7 Feb 2007

4. TITLE AND SUBTITLE

p63 in Development and Maintenance of the Prostate Epithelium

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-06-1-0365

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Sabina Signoretti, M.D.

Email: ssignoretti@partners.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Brigham and Women's Hospital
Boston, MA 02115

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES
Original contains colored plates: ALL DTIC reproductions will be in black and white.

14. ABSTRACT

The purpose of this project is to define the role of p63 in the development and maintenanceof the prostate epithelium by utilizing both in vivo and in vitro models.In our first year of work, we have the successful constructed the targeting vectorfor the generation of the p63-Cre-ERT2 knock-in mice to be used for genetic lineage tracing.The p63-Cre-ERT2 vector has been transferred to the DFCI Gene Targeting Core Facility andelectroporation in the ES cells will be performed shortly. The strategy for screening of EScells for homologous recombination has been already designed and probes for Southern Blotanalysis have been generated and successfully tested. In addition, we have made progress inexploring the molecular mechanisms through which p63 regulates development of the prostateepithelium. Specifically, the use of siRNA against p63 has been optimized in various celllines and, most importantly, p63 shRNA inducible cell lines (including iPrEC) have beengenerated. Preliminary results obtained utilizing these reagents show that downregulation ofp63 in iPrEC cells consistently causes a decrease in cell viability.

15. SUBJECT TERMS
p63, prostate, cancer, basal cells, stem cells, development

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT	b. ABSTRACT	c. THIS PAGE			
U	U	U	UU	13	19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

INTRODUCTION

Basic cancer research has focused on identifying the genetic alterations that cause cancer. This has led to major advances in our understanding of the molecular and biochemical pathways that are involved in tumorigenesis. However, since most of the work focused on the effects of particular molecular changes on the proliferation and survival of model cells, such as fibroblasts or cell lines, it is not clear what the effects of such changes will be on the actual cells involved in particular cancers. Thus, a clear understanding of the molecular abnormalities underlying the development of human malignancies, including prostate cancer, cannot be achieved without the identification of the cell type(s) involved in neoplastic transformation. Various cell types (i.e. secretory, intermediate, and stem cells) have been proposed as potential targets for prostate carcinogenesis [1-8]. However, a major limitation for the identification of the cell type(s) involved in the development and propagation of prostate cancer is that the identity of prostate stem cells and their differentiation programs remain unclear.

The scientific community has long debated the hierarchical relationship between basal and secretory cells in the prostate. The basal cell marker p63 is selectively expressed in the basal cells of several epithelia, including the prostate [9, 10]. We previously demonstrated that p63-deficient (p63-/-) mice present defects in prostate buds development [9]. Our recent work shows that when such developmental defects are abolished by complementing p63-/- blastocysts with p63+/+ ES cells, only p63+/+ cells compose the normal prostate epithelium of 7-weeks old chimeric mice [11]. These results indicate that prostate secretory cells of young adult mice derive from p63-positive progenitor cells that constitute the prostate buds. In addition, our UGS transplantation experiments show that p63 expression is required in progenitor cells to restrict development to the prostate cell lineage. As a whole, our preliminary data demonstrate that p63 is a key regulator of prostate development. On the basis of these data we hypothesize that 1) secretory cells forming throughout the entire lifespan derive from p63-positive stem/progenitor cells; 2) p63-positive cells of the adult prostate retain stem cell capabilities and thus function as adult stem cells; 3) p63 controls the development of prostate basal cells by regulating the expression of specific target genes.

Results from the proposed research project are likely to provide fundamental knowledge about the way the normal prostate epithelium develops and is renewed *in vivo*. Importantly, such knowledge is very critical for the advancement of the prostate cancer field.

Body

Research accomplishments based on the approved Statement of Work

Specific Aim 1: **To demonstrate that p63 is required for the development of secretory cells throughout the lifespan**.
We plan to utilize the p63-/-;ROSA26 chimera model that we recently developed. To demonstrate that secretory cells forming throughout the entire lifespan originate from p63-positive progenitors, the contribution of p63-/- and p63+/+ cells to the secretory cell compartment will be assessed and compared in p63-/-;ROSA26 chimeras sacrificed at different ages (7 weeks and 12 months).

We are working on the generation of the p63-/-;ROSA26 chimeras by injecting p63-/- ES cells into ROSA26 hemizygous blastocysts. We have been working on the selection of the optimal ES clones to be used for the generation of chimeric animals. We utilized real time quantitative PCR to determine both the genotype and the sex of 16 ES cell clones obtained from pre-implantation embryos (blastocysts) derived from p63+/- crosses. The karyotype of two p63+/- and two p63-/- male ES cell clones was subsequently determined in order to rule out the presence of chromosomal abnormalities. Results from these analyses are summarized in Table 1. Clone #1 (p63+/-) and clone #7 (p63-/-) were selected and are currently utilized for the generations of the p63-/-;ROSA26 and p63+/-;ROSA26 chimeras. A first experiment in which clone #1 and clone #7

ES cells ID	Sex	Genotype	Karyotype
1	M	Het	46 XY
2	M	Het	46 XY/92XXYY
3	M	Het	-
4	F	WT	-
5	M	Hom	-
6	M	Het	-
7	M	Hom	46 XY
8	M	Het	-
9	M	Het	-
10	M	Hom	46 XY
11	M	Het	-
12	F	Het	-
13	M	Hom	-
14	F	Hom	-
15	M	Het	-
16	F	Het	-

Table 1

have been injected into ROSA26 hemizygous blastocysts has been recently performed. Chimeric embryos will be analyzed by beta-gal staining at 18.5dpc.

Specific Aim 2: **To assess if p63-positive basal cells of the adult prostate sustain the renewal of secretory cells and thus represent/include adult prostate stem cells**.
This aim will be achieved by performing genetic lineage tracing experiments. We plan to generate mice expressing inducible Cre recombinase (Cre-ER^{T2}) under the control of the p63 promoter by knocking-in the Cre-ER^{T2} cDNA into the p63 locus. These mice will be then crossed with R26R reporter mice to generate double mutant p63- Cre-ER^{T2};R26R mice. Analysis of the prostate of the double mutant p63-Cre-ER^{T2};R26R mice after Tamoxifen administration will allow us to determine if p63-positive basal cells of the adult prostate sustain the renewal of secretory cells and thus function as adult
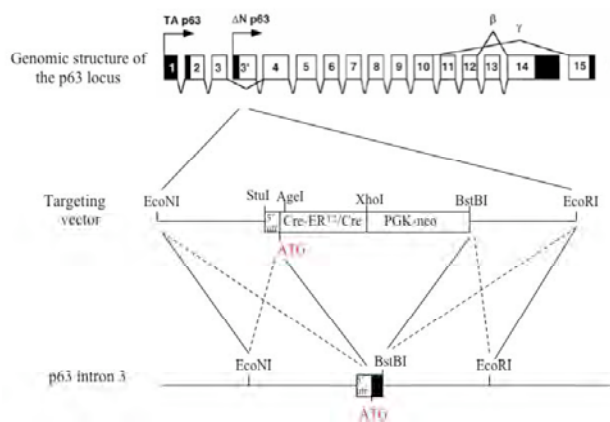

Fig.1 Strategy for knocking–in Cre-ER^{T2} and Cre in the ΔNp63 locus

prostate stem cells.

To date, the construction of the targeting vector for the generation of the p63-Cre-ER^{T2} knock-in mice has been completed. The p63-Cre-ER^{T2} vector is currently being electroporated in the ES cells.

1. Construction of the targeting vector

For the presence of two different promoters, located upstream of exon 1 and within intron 3, the p63 gene transcribes two isoforms, TA and ΔNp63. Since the ΔNp63alpha isoform is selectively expressed at high levels in basal cells of various epithelia, including the prostate, the p63-Cre-ER^{T2} knock-in mice are constructed by inserting a Cre-ER^{T2}-PGKneo cassette immediately downstream from the endogenous ΔNp63 promoter in intron 3. Specifically, after homologous recombination in the ES cells the start codon (ATG) of Cre-ER^{T2}/Cre will replace the start codon of the ΔNp63 transcript (Fig. 1).

The targeting constructs contain the diphtheria toxin A (DTA) cDNA, a homologous region upstream from the ΔNp63 ATG (5' arm), the Cre-ER^{T2}/Cre cDNA, the mouse neomycin phosphotransferase (neo) gene driven by the phosphoglycerate kinase (PGK) promoter, and a homologous region downstream from the ΔNp63 ATG (3' arm). The neo gene provides antibiotic resistance (neomycin) to the embryonic stem cells in which the homologous recombination has occurred successfully. This cassette is flanked by two FLP sites that will allow its excision from the ΔNp63 locus by crossing this mouse with an Frt mouse. The presence of the DTA cDNA is aimed at reducing the random genomic incorporation of the targeting construct.

The optimal homologous recombination rate is obtained by using ES cell lines derived from the 129 Sv/Ev mouse strain. Therefore, these ES cells are being used for the generation of the p63-Cre-ER^{T2} knock-in mice. Since even small gaps in homology due to sequence polymorphisms between mouse strains can dramatically reduce the efficiency of homologous recombination, the genomic DNA used for the construction of the targeting vectors was derived from the same strain of mouse as the ES cells, i.e. 129 Sv/Ev. To obtain the 129 Sv/Ev genomic clones containing the ΔNp63 locus, we screened a set of "dot-blot" membranes representing arrayed genomic clones of 129 Sv/Ev DNA in Bacterial Artificial Chromosomes (BAC), that have a 5-fold coverage of the entire genome. These membranes were obtained from the Dana-Farber Cancer Institute (DFCI) Gene Targeting facility directed by Dr. Ronald DePinho. Once the genomic clones containing the ΔNp63 locus were obtained, the targeting vector was constructed by the following steps:

1)   A 9.3kb fragment containing the 5' and 3' homologous recombination arms was excised from the BAC and inserted into the polylinker of plasmid pSL301, creating plasmid pSL301-9.3. The identity and structure of the ΔNp63 locus in the clone was verified by end-sequencing, restriction digestion, Southern Blot and PCR.

2)   A fragment of the 5' recombination arm (2861 bp) was subcloned from plasmid pSL301-9.3, into plasmid pKOII, creating plasmid pKOII-5A.

3)   A PGKNeo cassette was inserted into plasmid pKOII-5A, creating plasmid pKOII-5A.Neo. After subcloning, the structure of the inserted fragment was verified by thorough restriction enzyme digestion and full-length sequencing.

4)   A DNA fragment of 302 bp, including the downstream fragment of the 5' arm, was amplified by PCR using plasmid pSL301-9.3 as template. In order to replace the start codon (ATG) of the ΔNp63 transcript with the start codon of Cre-ER^{T2}, a sequence of 46 nucleotides, corresponding to the 5' sequence of Cre-ER^{T2} followed by the AgeI restriction site was included in the reverse primer. The PCR product was subcloned in the StuI-AgeI sites of pKOII-5A.Neo, creating pKOII-5B.NeoC. This step

completed the subcloning of the 5' recombination arm (3122 bp). Thorough restriction enzyme digestion and full-length sequencing verified the structure of the inserted fragment.

5) The fragment of Cre-ER^{T2} cDNA downstream from the AgeI site followed by a stop codon, was then subcloned in the AgeI-XhoI restriction sites of pKOII-5B.NeoC, creating pKOII-5B.NeoCre-ER^{T2}A. Restriction digestion and full-length sequencing verified the structure of the inserted fragment. This step completed the subcloning of the Cre-ER^{T2} cDNA.

6) Finally, the 3' recombination arm (3409 bp), was subcloned, in the NotI-SalI restriction sites of pKOII-5B.NeoCre-ER^{T2}A, creating the final targeting vector pKOII-5B.NeoCre-ER^{T2}B (13338 bp). Once completed, the structure of the targeting construct was verified by extensive restriction digestion and sequencing.

2. Screening of ES cells for homologous recombination

Electroporation of the linearized targeting vectors in the 129Sv/Ev ES cells is currently performed by the core facility directed by Dr. DePinho at DFCI.

The targeted ES clones that have correctly integrated the planned modifications by homologous recombination will be identified by both drug selection (neomycin and diphtheria toxin), and Southern blot

Fig.2 Southern Blot approach for screening of homologous recombination of the p63-Cre-ER^{T2} targeting vector in ES cells

analysis. Unique probes and restriction enzyme sites (SphI) that lie outside the homology regions will be used in Southern blot analysis to identify correctly recombined neomycin resistant ES clones (Fig. 2).
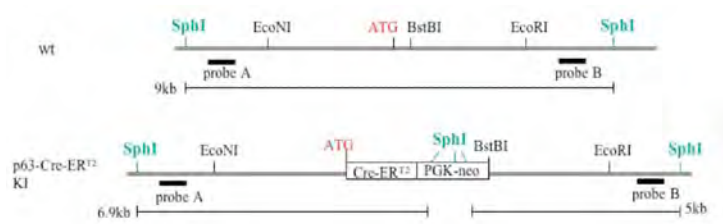
Specific Aim 3: To identify p63 target genes mediating p63 function in prostate development.

To identify the molecular mechanisms through which p63 regulates development of the prostate epithelium, we are utilizing immortalized prostate epithelial cells (iPrEC) obtained from Dr. William Hahn laboratory at DFCI. Other cell lines, including MCF 10A (immortalized breast epithelial cells), SCC 9 and SCaBER (squmaous cell carcinoma cell lines) are also utilized. We have worked on silencing the expression of endogenous p63 in iPrEC as well as other cell lines cells using RNA interference. The effects of p63 silencing on cell proliferation, cell death, and activation of various signaling pathways is currently being assessed.

1. Development of siRNA against p63: We designed two oligo siRNAs against p63: 1) Oligo 3 siRNA, which knocks down all the p63 isoforms 2) DNp63 siRNA, which is designed in the untranslated region of DNp63 and specifically knocks down only DNp63 isoforms.

In our preliminary experiments, we optimized the concentration of the oligo siRNA to get efficient knock down of the p63 protein levels. We assayed different concentrations of the siRNA and found that 40 nM siRNA (both Oli3 and DNp63 siRNAs) induces a 80% knock down in p63 levels compared to the control siRNA. In all our experiments using oligo siRNA, we used scramble siRNA (Dharmacon) as a control. For the transfection of siRNA in SCC 9 cell line and MCF 10A we used Oligofectamine and Lipofectamine 2000 (Invitrogen), respectively. Unfortunately,
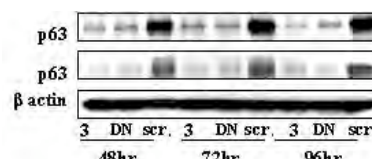

Fig 3. Validation of siRNA. Oligo3, DNp63 and scramble siRNA at 40 nM conc. are transfected in SCC9 (upper panel) and MCF 10A (lower panel) cells and probed for p63.

iPrEC cell could not be efficiently transfected with liposomal transfection reagents. By using the Amaxa's Nucleofector technology, we were also unsuccessful in silencing p63 expression iPrEC cell. In order to overcome this problem, we switched to a Tetracycline-inducible lentiviral siRNA expression system. Specifically, we used pBLOCK-iT inducible RNAi lentiviral expression system from Invitrogen. This system has several advantages including: a) Lentiviral transduction is very efficient in the cell lines that are difficult to transfect with other protocols b) the use of Tet-inducible clones allows a relatively easy manipulation of the experimental strategy.

2. Generation of p63 shRNA inducible cell lines.

The mechanism of tetracycline regulation in the system is based on the binding of tetracycline to the Tet repressor and derepression of the promoter controlling expression of the shRNA of interest. In the system, expression of short hairpin RNA (shRNA) is repressed in the absence of tetracycline and induced in its presence.

We constructed the Tet-inducible cell line by the following procedure:

a. We designed the shRNAs based on the sequences of oligo3 siRNA and DNp63 siRNA.

b. We inserted Oli3 and DNp63 shRNAs in pENTER/H1/TO entry vector and screened for the efficiency of the knockdown by measuring p63 proteins levels after transient transfection in SCC 9 cell line. p63 protein levels were assesses at 72h and 96h time points by western blotting (Fig. 2).

Fig 4. Validation of shRNA. Oligo3 shRNA and DNp63 shRNA are transfected in SCC 9 cells lines p63 levels were tested by western blotting after 96hr

c. For developing stable clones expressing shRNA, we recombined the pENTR/H1/TO-oli3 and pENTR/H1/TO-DNp63 entry constructs with pLenti4/BLOCK-IT DEST to generate pLenti4/BLOCK-IT-Oli3 and pLenti4/BLOCK-IT-DNp63 shRNA constructs.

d. Generation of Tet repressor expressing cell lines: For the tight regulation of the expression of shRNA, high levels expression of Tet repressor (Tet R) is crucial. We transduced the cell lines SCC 9, SCaBER and iPrEC with the pLenti6/TR lentiviral expression construct and generated the stable clones by taking the advantage of the antibiotic resistance (Blasticidin). We screened for the clones with the high levels of Tet R protein and clones with high expression levels were selected and named accordingly (SCC9 TR, SCABER TR, iPrEC TR).

Fig 5. Tet repressor expression.. Tet R levels were measured by in SCC 9 by western blotting against positive control after transduction with pLenti/TR construct

Fig 6. Screeing of Tet-inducible clones. a) SCC 9, b) iPrEC, c) SCaBER cell lines. Clones were treated with Tetracycline for 96 hr and immunoprobed for p63

e. Finally, we generated the Tet- inducible cells lines by infecting SCC9 TR, SCaBER TR and iPrEC TR, with pLenti4/BLOCK-IT-Oli3 and pLenti4/BLOCK-IT-DNp63 shRNA constructs. We isolated the clones by antibiotic selection (zeocin) and screened for those showing at least 50% knock down upon addition of tetracycline (5ug/ml) for 72 hr.
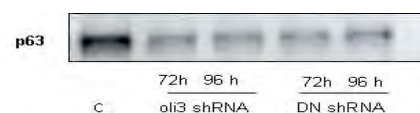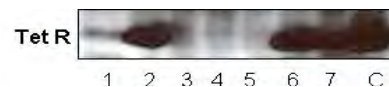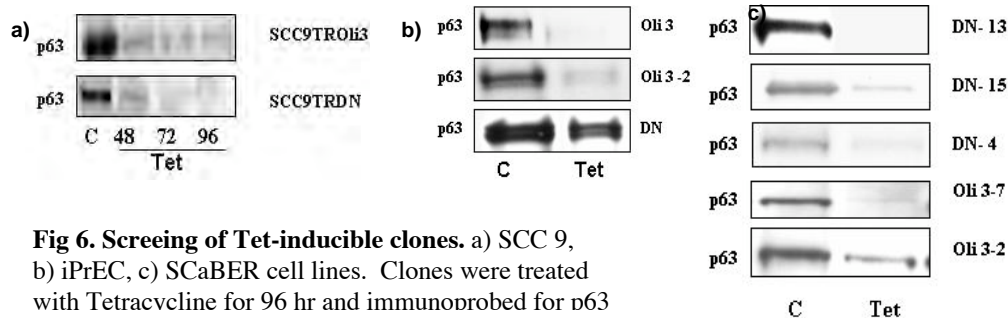
8

3. Cell viability assay.

In our initial observations, we observed an important decrease in the cell number in with p63 knockdown as compared to controls. To quantify the effect of p63 downregulation on cell viability, we utilized the MTT assay. We assessed the cell viability at different time points after p63 silencing in SCC 9, SCaBER, and MCF 10A cell lines. In all the cell lines, there was a significant decrease in the cell viability with both Oligo3 siRNA and DNp63 siRNA compared to scramble. Similarly, p63 knockdown in inducible shRNA iPrEC clones showed a significant decrease in cell viability.
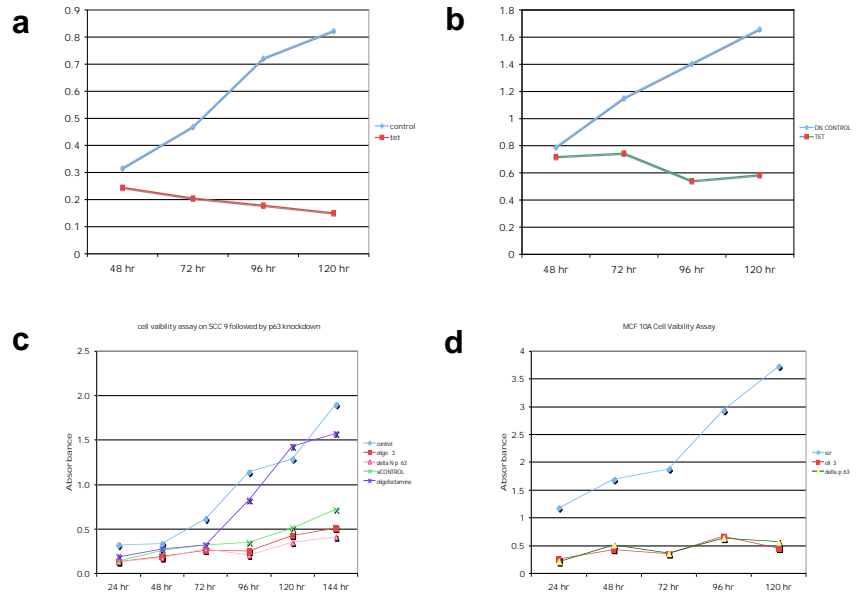
Fig 7. Cell Vaibility Assay. a) iPrEC oli3 clone, b) iPrEC DN clone c) SCC 9 cell line d) MCF 10A cell line. a-b)iPrEC clones were treated With Tetracycline and cell vaibility was determined by MTT assay at 48, 72, 96 and 120h. c-d)SCC 9 and MCF 10A cells were transfected with oli3, DNp63 and Scr siRNA and cell vaibility Was measured at 24, 48, 72, 96, and 120 hr.

9

KEY RESEARCH ACCOMPLISHMENTS

Aim 1
a. We have been working on the selection of the optimal p63-/- ES clones to be used for the generation of p63-/-;ROSA26 chimeras.

Aim 2
a. The construction of the targeting vector for the generation of the p63-Cre-ER^{T2} knock-in mice has been completed.
b. The p63-Cre-ER^{T2} vector is currently being electroporated in the ES cells
c. The strategy for screening of ES cells for homologous recombination has been designed. Probes for Southern Blot analysis have been generated and tested.

Aim 3
a. The use of siRNA against p63 has been optimized in various cell lines.
b. p63 shRNA inducible cell lines (including iPrEC) have been generated.
c. Downregulation of p63 in iPrEC cells (and other cell lines) consistently results in a decrease in cell viability

REPORTABLE OUTCOMES

Manuscripts sponsored by the W81XWH-06-1-0365 award:

1. **Signoretti S*** and Loda M*. Defining stem cells in the prostate epithelium. Cell Cycle. 2006; 5:138-41.

2. A M De Marzo , E A Platz , J I Epstein , T Ali , A Billis, T Y Chan , L Cheng, M Datta, L Egevad, D Ertoy-Baydar , X Farre , S W Fine , K A Iczkowski, M Ittmann, B S Knudsen, M Loda, A Lopez-Beltran, C Magi-Galluzzi, G Mikuz , R Montironi, E Pikarsky, G Pizov, M A Rubin, H Samaratunga, T Sebo, I A Sesterhenn, RB Shah, **S Signoretti**, J Simko ,G Thomas, P Troncoso, T Tsuzuki, G JLH van Leenders, XJ Yang, M Zhou, W D Figg, A Hoque and M S Lucia. A working group classification of focal prostate atrophy lesions. Am J Surg Pathol. 2006 Oct;30(10):1281-91.

3. Berger R, Lin DI, Nieto, Sicinska Ewa, L. Garraway LA, Heiner A, **Signoretti S,** Hahn WC and Loda M. Androgen-dependent regulation of Her-2/neu In Prostate Cancer Cells. Cancer Res 2006; 66:5723-8.

4. **Signoretti S*** and Loda M*. Prostate stem cells: from development to cancer. Semin Cancer Biol. 2006 May 10

5. Zhan Q, **Signoretti S**, Whitaker-Menezes D, Friedman TM, Korngold R and Murphy GF. Cytokeratin15-Positive Basal Epithelial Cells Targeted in Graft-Versus-Host Disease Express a Constitutive Antiapoptotic Phenotype. J Invest Dermatol. 2006 Oct 12.

* Co-corresponding authors.

CONCLUSIONS

In our first year of work, we have the successful constructed the targeting vector for the generation of the p63-Cre-ER^{T2} knock-in mice. The p63-Cre-ER^{T2} vector has been transferred to the DFCI Gene Targeting Core Facility and electroporation in the ES cells will be performed shortly. The strategy for screening of ES cells for homologous recombination has been already designed and probes for Southern Blot analysis have been generated and successfully tested.

In addition, we have made progress in exploring the molecular mechanisms through which p63 regulates development of the prostate epithelium. Specifically, the use of siRNA against p63 has been optimized in various cell lines and, most importantly, p63 shRNA inducible cell lines (including iPrEC) have been generated. Preliminary results obtained utilizing these reagents show that downregulation of p63 in iPrEC cells consistently causes a decrease in cell viability.

REFERENCES

1. Liu, A.Y., et al., *Cell-cell interaction in prostate gene regulation and cytodifferentiation*. Proc Natl Acad Sci U S A, 1997. **94**(20): p. 10705-10.
2. Nagle, R.B., et al., *Cytokeratin characterization of human prostatic carcinoma and its derived cell lines*. Cancer Res, 1987. **47**(1): p. 281-6.
3. Colombel, M., et al., *Detection of the apoptosis-suppressing oncoprotein bc1-2 in hormone-refractory human prostate cancers*. Am J Pathol, 1993. **143**(2): p. 390-400.
4. McDonnell, T.J., et al., *Expression of the protooncogene bcl-2 in the prostate and its association with emergence of androgen-independent prostate cancer*. Cancer Res, 1992. **52**(24): p. 6940-4.
5. Pisters, L.L., et al., *c-met proto-oncogene expression in benign and malignant human prostate tissues*. J Urol, 1995. **154**(1): p. 293-8.
6. Reiter, R.E., et al., *Prostate stem cell antigen: a cell surface marker overexpressed in prostate cancer*. Proc Natl Acad Sci U S A, 1998. **95**(4): p. 1735-40.
7. Signoretti, S., et al., *Her-2-neu expression and progression toward androgen independence in human prostate cancer*. J Natl Cancer Inst, 2000. **92**(23): p. 1918-25.
8. Verhagen, A.P., et al., *Differential expression of keratins in the basal and luminal compartments of rat prostatic epithelium during degeneration and regeneration*. Prostate, 1988. **13**(1): p. 25-38.
9. Signoretti, S., et al., *p63 is a prostate basal cell marker and is required for prostate development*. Am J Pathol, 2000. **157**(6): p. 1769-75.
10. Yang, A., et al., *p63, a p53 homolog at 3q27-29, encodes multiple products with transactivating, death-inducing, and dominant-negative activities*. Mol Cell, 1998. **2**(3): p. 305-16.
11. Signoretti, S., et al., *p63 regulates commitment to the prostate cell lineage*. Proc Natl Acad Sci U S A, 2005. **102**(32): p. 11355-60.